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FOR: **A METHOD FOR DETECTION OF CARCINOEMBRYONIC ANTIGENS HAVING A MODIFIED SUGAR CHAIN STRUCTURE**

CLAIM FOR PRIORITY UNDER 35 U.S.C. 119

Commissioner for Patents
Washington, D.C. 20231

August 12, 2002

Sir:

The benefit of the filing date of the following prior foreign application is hereby requested for the above-identified application, and the priority provided in 35 U.S.C. 119 is hereby claimed:

Japanese Appln. No. 11-172485, filed June 18, 1999

In support of this claim, the requisite certified copy of said original foreign application is filed herewith.

It is requested that the file of this application be marked to indicate that the applicants have complied with the requirements of 35 U.S.C. 119 and that the Patent and Trademark Office kindly acknowledge receipt of said certified copy.

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2340.

Respectfully submitted,

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出証特２０００－３０４８２０８

【書類名】　明細書

【発明の名称】　糖鎖構造の変異した癌胎児性抗原類の検出方法

【特許請求の範囲】

【請求項１】癌胎児性抗原（以下、ＣＥＡと略記する。）類の不変領域に特異的な抗体（以下、ＣＥＡ類結合抗体と略記する。）と、ＣＥＡ類の変異した糖鎖構造を認識する蛋白質（以下、特定糖鎖結合蛋白質と略記する。）とを用いることを特徴とする、糖鎖構造の変異したＣＥＡ類の検出方法。

【請求項２】ＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の有無を測定することを特徴とする、請求項１に記載の検出方法。

【請求項３】試料と、ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質とを反応させ、生成したＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の有無を測定する、請求項１に記載の検出方法。

【請求項４】特定糖鎖結合蛋白質が抗体又はレクチンである、請求項１～３の何れかに記載の検出方法。

【請求項５】抗体がフコース残基又は／及びシアル酸残基の存在を認識する抗体である、請求項４に記載の検出方法。

【請求項６】抗体が抗ルイス抗体又は抗シアリルルイス抗体である、請求項４に記載の検出方法。

【請求項７】抗ルイス抗体が抗Ｌｅ^{a}抗体、抗Ｌｅ^{b}抗体、抗Ｌｅ^{x}抗体又は抗Ｌｅ^{y}抗体である、請求項６に記載の検出方法。

【請求項８】抗シアリルルイス抗体が抗Ｓ-Ｌｅ^{a}抗体又は抗Ｓ－Ｌｅ^{x}抗体である、請求項６に記載の検出方法。

【請求項９】レクチンがＬ－フコース結合性レクチン、Ｄ－ガラクトース又はＮ－アセチル－Ｄ－ガラクトサミン結合性レクチン、Ｄ－マンノース結合性レクチン、Ｎ－アセチルグルコサミン結合性レクチン又はシアル酸結合性レクチンである、請求項４に記載の検出方法。

【請求項１０】レクチンがコンカナバリンＡ、ヒマレクチン、レンズマメレクチン又はインゲンマメレクチンである、請求項４に記載の検出方法。

【請求項１１】糖鎖構造の変異したＣＥＡ類の量に基づいて判定を行う、癌の判

定方法。

【請求項１２】ＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の量を測定し、この値に基づいて判定を行う請求項１１に記載の癌の判定方法。

【請求項１３】ＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の量と、ＣＥＡ類とＣＥＡ類結合抗体との複合体の量とを測定し、これらの値に基づいて判定を行う請求項１１に記載の判定方法。

【請求項１４】ＣＥＡ類を含む試料と、ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質とを反応させ、生成したＣＥＡ類とＣＥＡ類結合抗体との複合体の量及びＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の量とを測定し、これらの値に基づいて判定を行う請求項１１に記載の判定方法。

【請求項１５】ＣＥＡ類を含む試料と、ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質とを反応させ、生成したＣＥＡ類とＣＥＡ類結合抗体との複合体の量、及びＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の量を夫々測定し、これら複合体の総量に対する後者の複合体の割合を求め、この値に基づいて判定を行う請求項１１に記載の判定方法。

【請求項１６】特定糖鎖結合蛋白質が抗体又はレクチンである、請求項１２～１５の何れかに記載の判定方法。

【請求項１７】抗体がフコース残基又は／及びシアル酸残基の存在を認識する抗体である、請求項１６に記載の判定方法。

【請求項１８】抗体が抗ルイス抗体又は抗シアリルルイス抗体である、請求抗１６に記載の判定方法。

【請求項１９】抗ルイス抗体が抗Ｌｅ^{a}抗体、抗Ｌｅ^{b}抗体、抗Ｌｅ^{x}抗体又は抗Ｌｅ^{y}抗体である、請求項１８に記載の判定方法。

【請求項２０】抗シアリルルイス抗体が抗Ｓ-Ｌｅ^{a}抗体又は抗Ｓ－Ｌｅ^{x}抗体である、請求項１８に記載の判定方法。

【請求項２１】レクチンがＬ－フコース結合性レクチン、Ｄ－ガラクトース又はＮ－アセチル－Ｄ－ガラクトサミン結合性レクチン、Ｄ－マンノース結合性レクチン、Ｎ－アセチルグルコサミン結合性レクチン又はシアル酸結合性レクチンである、請求項１６に記載の判定方法。

【請求項２２】レクチンがコンカナバリンＡ、ヒマレクチン、レンズマメレクチン又はインゲンマメレクチンである、請求項１６に記載の判定方法。

【請求項２３】ＣＥＡ類結合抗体と特定糖鎖結合蛋白質とを含有させて成る、糖鎖の変異したＣＥＡ類の検出用キット。

【請求項２４】特定糖鎖結合蛋白質が抗体又はレクチンである、請求項２３に記載のキット。

【請求項２５】抗体がフコース残基又は／及びシアル酸残基の存在を認識する抗体である、請求項２４に記載のキット。

【請求項２６】抗体が抗ルイス抗体又は抗シアリルルイス抗体である、請求抗２４に記載のキット。

【請求項２７】抗ルイス抗体が抗Ｌｅ^{a}抗体、抗Ｌｅ^{b}抗体、抗Ｌｅ^{x}抗体又は抗Ｌｅ^{y}抗体である、請求項２６に記載のキット。

【請求項２８】抗シアリルルイス抗体が抗Ｓ-Ｌｅ^{a}抗体又は抗Ｓ－Ｌｅ^{x}抗体である、請求項２６に記載のキット。

【請求項２９】レクチンがＬ－フコース結合性レクチン、Ｄ－ガラクトース又はＮ－アセチル－Ｄ－ガラクトサミン結合性レクチン、Ｄ－マンノース結合性レクチン、Ｎ－アセチルグルコサミン結合性レクチン又はシアル酸結合性レクチンである、請求項２４に記載のキット。

【請求項３０】レクチンがコンカナバリンＡ、ヒマレクチン、レンズマメレクチン又はインゲンマメレクチンである、請求項２４に記載のキット。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】

本発明は、癌胎児性抗原（carcinoembryonic antigen、以下、「ＣＥＡ」と略記する。）の検出方法に関するものであり、特に糖鎖構造の変異したＣＥＡ類の検出方法及びキットに関する。また、本発明は、糖鎖構造の変異したＣＥＡ類の量を求め、この量に基づいて判定を行う、癌の判定方法に関する。

【０００２】

【従来の技術】

ＣＥＡは、癌細胞から産生される胎児性抗原の一つであり、正常消化器粘膜細胞表面および腫瘍組織から産生され、分子量２０万前後、糖含量が約５０％の糖蛋白質の一種である。ＣＥＡは、正常ヒトの血中には存在しないが、大腸癌、肺癌、胃癌、乳癌、肝癌などの臓器癌になると、細胞中や血中の濃度が上昇することが知られている。そのため、ＣＥＡ量は広範囲の腫瘍マーカーとして有用であり、血中のＣＥＡ量の測定は、癌のスクリーニングや術後の経過観察、再発予防に広く用いられている。しかしながら、血中のＣＥＡ量の測定だけでは早期癌の鑑別は困難であった。

そのため、腫瘍組織の早期癌の鑑別には、Ｘ線診断、内視鏡的診断等がおこなわれている。しかし、Ｘ線診断では早期癌を発見するのは困難であり、内視鏡的診断では診断技術と知識の程度によって診断が左右されるという問題がある。

【０００３】

一方、ＣＥＡ類産生細胞である正常消化器粘膜細胞および腫瘍細胞よりＣＥＡ類を夫々精製し、その糖鎖構造を解析した結果、正常消化器粘膜細胞と腫瘍細胞とでは結合している糖鎖の構造が違っていることが判明し、癌の診断に有用ではないかと期待された（Katsuko Yamashita,J.Biol.Chem.,264(30),17873-17881(1989)、Katsuko Yamashita,Glycobiology,5(1),105-115(1995)）。しかしこの方法では、採取した細胞からＣＥＡ類を精製したり、その糖鎖構造を解析しなければならないため、診断までに長時間を要するという問題があった。

【０００４】

【発明が解決しようとする課題】

以上のような状況から、本発明が解決しようとする課題は、容易に且つ簡便に生体由来試料中の各種ＣＥＡ類を検出できる方法、この測定結果に基づき癌を鑑別し得る方法並びにそのために用いられる試薬の提供にある。

【０００５】

【課題を解決するための手段】

本発明は、このような課題を解決するために成されたものであり、

（１）ＣＥＡ類の不変領域に特異的な抗体（以下、「ＣＥＡ類結合抗体」と略記する。）と、ＣＥＡ類の変異した糖鎖構造を認識する蛋白質（以下、「特定糖鎖

結合蛋白質」と略記する。）とを用いることを特徴とする、糖鎖の変異したＣＥＡ類の検出方法、

（２）糖鎖構造の変異したＣＥＡ類の量に基づいて判定を行う、癌の判定方法、

（３）ＣＥＡ類結合抗体と、特定糖鎖結合蛋白質を含有させて成る、糖鎖の変異したＣＥＡ類の検出用キット、に関する。

【０００６】

即ち、本発明者らは、上記した如き課題を解決するために鋭意研究した結果、ＣＥＡ類結合抗体と、特定糖鎖結合蛋白質を夫々１以上用いることにより、生体試料中のＣＥＡ類の総量、特定の変異した糖鎖構造を有するＣＥＡ類量又は／及びそれ以外の糖鎖構造を有するＣＥＡ類量を測定し得ることを見出し、更に研究を重ねた結果、特定の変異した糖鎖構造を有するＣＥＡ類量又はそれ以外の糖鎖構造を有するＣＥＡ類量、或いは全ＣＥＡ類中の、特定の変異した糖鎖構造を有するＣＥＡ類又はそれ以外の糖鎖構造を有するＣＥＡ類の割合が、例えば大腸癌等の判別に有用であることを見出し、本発明を完成するに到った。

【０００７】

本発明に係るＣＥＡ類結合抗体としては、ＣＥＡ類の不変領域に特異的な性質を有する抗体であれば特に限定されず、常法、例えば［免疫実験学入門、第２刷、松橋直ら、（株）学会出版センター、1981］等に記載の方法に従って、例えば馬、牛、羊、兎、山羊、ラット、マウス等の動物に測定対象を免役して作製されるポリクローナル性抗体でも、或いはまた常法、即ちケラーとミルスタイン（Nature、256巻,495頁,1975）により確立された細胞融合法に従って、例えばマウスの腫瘍ラインからの細胞と測定対象物で予め免役されたマウスの脾臓細胞を融合させて得られるハイブリドーマが産出する単クローン性抗体でもよい。また、これらは単独で用いても、適宜組み合わせて用いてもよい。尚、ＣＥＡ類の不変領域とは、生体試料中の全てのＣＥＡ類に共通な構造領域のことを指す（MASAHIDE KUROKI,HYBRIDOMA,4(11),391-407(1992)）。

【０００８】

ＣＥＡ類結合抗体には、変異した糖鎖構造を有するＣＥＡ類の当該変異した糖鎖構造に特異的に結合する蛋白質（特定糖鎖結合蛋白質）が結合したＣＥＡ類と

は結合しない性質を有するＣＥＡ類結合抗体（以下、「競合性ＣＥＡ類結合抗体」と略記する。）や特定糖鎖結合蛋白質の結合の有無に拘わらず、全てのＣＥＡ類と結合し得る性質を有するＣＥＡ類結合抗体（以下、「非競合性ＣＥＡ類結合抗体」と略記する。）等が含まれる。

【０００９】

本発明に係る特定糖鎖結合蛋白質としては、例えばＣＥＡ類の特定糖鎖構造に特異的に結合する抗体、レクチンなどが挙げられる。具体的には、フコース残基又は／及びシアル酸残基の存在を認識する抗体、更に具体的には、例えばＬｅ^{a}，Ｌｅ^{b}，Ｌｅ^{x}，Ｌｅ^{y}等のルイス型糖鎖を認識する抗ルイス抗体，Ｓ－Ｌｅ^{a}，Ｓ－Ｌｅ^{X}等のシアリルルイス型糖鎖を認識する抗シアリルルイス抗体等の抗体類、例えばミヤコグサレクチン等のＬ－フコース結合性レクチン、例えばピーナッツレクチン，ダイズレクチン，ヒマレクチン，インゲンマメレクチン等のＤ－ガラクトース又はＮ－アセチル－Ｄ－ガラクトサミン結合性レクチン、例えばコンカナバリンＡ，レンズマメレクチン，エンドウマメレクチン等のＤ－マンノース結合性レクチン、例えば小麦胚芽レクチン，ダツラレクチン等のＮ－アセチルグルコサミン結合性レクチン、例えばカブトガニレクチン等のシアル酸結合性レクチン等のレクチン類が挙げられる。中でもＤ－ガラクトース又はＮ－アセチル－Ｄ－ガラクトサミン結合性レクチン、Ｄ－マンノース結合性レクチン等が好ましい。また、これらは単独で用いても、適宜組み合わせて用いてもよい。

【００１０】

尚、上記のレクチンの分類に於いて、結合性とは、適当な糖鎖を結合させたアフィニティーカラムに一旦結合させたレクチンがどのような糖で溶出され易いかを示すもので、例えばＤ－ガラクトース又はＮ－アセチル－Ｄ－ガラクトサミン結合性レクチンとは、アフィニティーカラムに一旦結合させた後、Ｄ－ガラクトース若しくはＮ－アセチル－Ｄ－ガラクトサミンにより溶出されるレクチンのことを意味する。

【００１１】

また、変異した特定の糖鎖構造を特異的に認識する抗体も、上記した如き常法に準じて調製されたポリクローン性抗体でも単クローン性抗体でもよい。

【００１２】

本発明において、特定糖鎖構造とは、より具体的には、①上記した如きレクチンが結合し得る糖鎖構造、②例えば大腸癌細胞等の腫瘍細胞が産生するＣＥＡ類が有する糖鎖構造、等であり、更に具体的には例えばYamamoto, K., Eur. J. Biochem.,143(1),133-144,1984等の文献に記載された如き糖鎖構造である。

【００１３】

本発明に係る糖鎖構造の変異したＣＥＡ類の検出方法としては、ＣＥＡ類結合抗体と特定糖鎖結合蛋白質とを適宜組み合わせて、例えばＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の有無を測定することにより行う。

【００１４】

本発明に係る癌の判定方法は、例えば糖鎖構造の変異したＣＥＡ類の量に基づいて判定を行う方法であり、具体的には、①上記方法により求めた、ＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体の量に基づいて判定を行う方法、②ＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体（糖鎖構造の変異したＣＥＡ類）の量と、ＣＥＡ類とＣＥＡ類結合抗体との複合体（それ以外の糖鎖構造を有するＣＥＡ類）の量に基づいて判定を行う方法、又は③ＣＥＡ類とＣＥＡ類結合抗体との複合体の量（それ以外の糖鎖構造を有するＣＥＡ類）及びＣＥＡ類とＣＥＡ類結合抗体と特定糖鎖結合蛋白質との複合体（糖鎖構造の変異したＣＥＡ類）の量を夫々求め、これら複合体の総量に対する後者の複合体の割合、換言すればＣＥＡ類結合抗体及び特定糖鎖結合蛋白質と複合体を形成したＣＥＡ類（糖鎖構造の変異したＣＥＡ類）の割合を求めて、この値に基づいて判定を行う方法等が挙げられる。

【００１５】

即ち、その具体的な測定対象は、例えば総ＣＥＡ類、変異した特定の糖鎖構造を有するＣＥＡ類及びそれ以外の糖鎖構造を有するＣＥＡ類等である。尚、ＣＥＡ類は、生体中で分解作用により、種々のフラグメントになるが、このようなものであってもＣＥＡ類結合抗体又は／及び特定糖鎖結合蛋白質が結合し得るものは、本発明の測定対象である。これら測定対象は、別々に測定してもよいし、一度の測定で同時に測定してもよい。

【００１６】

更に具体的に述べれば、例えばレクチン又は抗ＣＥＡ抗体等の特定糖鎖結合蛋白質を用いて、総ＣＥＡ類中の、特定の糖鎖構造を有するＣＥＡ類又はそれ以外の糖鎖構造を有するＣＥＡ類の割合を求め、その値を正常の試料を用いて得られた値と比較すれば、癌の判別が可能となる。本方法は、本発明者らが初めて見出したものである。

以下にＣＥＡ類の測定方法の具体例を述べる。

【００１７】

Ｉ．測定対象を別々に測定する方法。

測定対象、即ち、例えば総ＣＥＡ類、変異した特定の糖鎖構造を有するＣＥＡ類、それ以外の糖鎖構造を有するＣＥＡ類等は、夫々例えば以下のようにして測定される。

【００１８】

Ｉ－１．総ＣＥＡ類の測定

ＣＥＡ類結合抗体を用いる自体公知の測定法により測定すればよい。

【００１９】

Ｉ－２．変異した特定の糖鎖構造を有するＣＥＡ類の測定

Ｉ－２－１）ＣＥＡ類結合抗体固定化不溶性担体を用いる方法。

例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、ＣＥＡ類結合抗体固定化不溶性担体とを反応させて、下記の固定化複合体を形成させる。

［不溶性担体］－ＣＥＡ類結合抗体－ＣＥＡ類

次いで、不要な共存物質を洗浄等で除去した後、当該固定化複合体に、更に標識物質が結合した特定糖鎖結合蛋白質（以下、「標識特定糖鎖結合蛋白質」と略記する。）を反応させて、下記の固定化複合体を形成させる。

［不溶性担体］－ＣＥＡ類結合抗体－ＣＥＡ類－標識特定糖鎖結合蛋白質

次いで、当該固定化複合体を洗浄する等して遊離の標識特定糖鎖結合蛋白質を除去した後、当該固定化複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の特定糖鎖構造を有するＣＥＡ類を含む標

準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）とＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の特定糖鎖構造を有するＣＥＡ類量を求めることができる。

【００２０】

Ｉ－２－２）特定糖鎖結合蛋白質固定化不溶性担体を用いる方法。

例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、特定糖鎖結合蛋白質固定化不溶性担体とを反応させて、下記の固定化複合体を形成させる。

［不溶性担体］－特定糖鎖結合蛋白質－ＣＥＡ類

次いで、不要な共存物質を洗浄等で除去した後、当該固定化複合体に、更に標識物質が結合したＣＥＡ類結合抗体（以下、「標識ＣＥＡ類結合抗体」と略記する。）を反応させて、下記の固定化複合体を形成させる。

［不溶性担体］－特定糖鎖結合蛋白質－ＣＥＡ類－標識ＣＥＡ類結合抗体

次いで、当該固定化複合体を洗浄する等して遊離の標識ＣＥＡ類結合抗体を除去した後、当該固定化複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の特定糖鎖構造を有するＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）とＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の特定糖鎖構造を有するＣＥＡ類量を求めることができる。

【００２１】

Ｉ－２－３）標識特定糖鎖結合蛋白質と高速液体クロマトグラフィー（ＨＰＬＣ）等とを用いる方法。

例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、標識特定糖鎖結合蛋白質と非競合性ＣＥＡ類結合抗体とを反応させて、試料中に下記の複合体を形成させる。

標識特定糖鎖結合蛋白質－ＣＥＡ類－非競合性ＣＥＡ類結合抗体

次いで、この複合体と遊離の標識特定糖鎖結合蛋白質とを、適当な充填剤を充填したＨＰＬＣや電気泳動法等を用いて分離し、該複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の特定糖鎖構造

を有するＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）とＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の特定糖鎖構造を有するＣＥＡ類量を求めることができる。

【００２２】

Ｉ－３．特定の糖鎖構造以外の糖鎖構造を有するＣＥＡ類の測定

Ｉ－３－１）遊離の特定糖鎖結合蛋白質を用いる方法

先ず、例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、特定糖鎖結合蛋白質とを反応させ、試料中の特定糖鎖構造を有するＣＥＡ類と特定糖鎖結合蛋白質との複合体（以下、「糖鎖結合ＣＥＡ類」と略記する場合がある。）を生成させる。次いで、糖鎖結合ＣＥＡ類と、特定糖鎖結合蛋白質が結合しなかったＣＥＡ類、言い換えれば、特定の糖鎖構造以外の糖鎖構造を有するＣＥＡ類（以下、「非結合ＣＥＡ類」と略記する場合がある。）とを、例えば、遠心分離法、ゲル濾過法、分子分画膜法、電気泳動法等の自体公知の分離方法を利用して試料から分離することにより非結合ＣＥＡ類のみを含む試料を調製する。

このようにして得られた、非結合ＣＥＡ類のみを含む試料中のＣＥＡ類量を、ＣＥＡ類結合抗体を用いる自体公知の測定法により測定することにより、非結合ＣＥＡ類量を求めることができる。

尚、非結合ＣＥＡ類のみを含有する試料は、特定糖鎖結合蛋白質を固定化した担体を用いるアフィニティクロマトグラフィーによって試料を処理することにより調製してもよい。

【００２３】

Ｉ－３－２）競合性ＣＥＡ類結合抗体を用いる方法

先ず、ＣＥＡ類結合抗体を固定化した不溶性担体と、例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料とを反応させ、下記の固定化複合体を形成させる。

［不溶性担体］－ＣＥＡ類結合抗体－ＣＥＡ類

次いで、不要な共存物質を洗浄等で除去した後、当該固定化複合体に特定糖鎖

結合蛋白質を反応させ、更に適当な標識物質を結合させた競合性ＣＥＡ類結合抗体（以下、「標識競合性ＣＥＡ類結合抗体」と略記する。）を反応させて、下記の固定化複合体を形成させる。

［不溶性担体］－ＣＥＡ類結合抗体－ＣＥＡ類－特定糖鎖結合蛋白質

［不溶性担体］－CEA類結合抗体－CEA類－標識競合性CEA類結合抗体

次いで、当該固定化複合体を洗浄する等して遊離の標識競合性ＣＥＡ類結合抗体を除去した後、当該固定化複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の特定糖鎖構造以外の糖鎖構造を有するＣＥＡ類、即ち、非結合ＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）とＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の非結合ＣＥＡ類量を求めることができる。

【００２４】

Ｉ－３－３）競合性ＣＥＡ類結合抗体固定化不溶性担体を用いる方法。

先ず、例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、特定糖鎖結合蛋白質とを反応させ、試料中に糖鎖結合ＣＥＡ類を生成させる。次いで、この試料と、競合性ＣＥＡ類結合抗体固定化不溶性担体とを反応させて下記の固定化複合体を生成させる。

［不溶性担体］－競合性ＣＥＡ類結合抗体－非結合ＣＥＡ類

次いで、不要な共存物質を洗浄等で除去した後、当該固定化複合体に標識ＣＥＡ類結合抗体を反応させて、下記の固定化複合体を形成させる。

［不溶性担体］－競合性CEA類結合抗体－非結合CEA類－標識CEA類結合抗体

次いで、当該固定化複合体を洗浄する等して遊離の標識ＣＥＡ類結合抗体を除去した後、当該固定化複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の非結合ＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）とＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の非結合ＣＥＡ類量を求めることができる。

【００２５】

Ｉ－３－４）標識競合性ＣＥＡ類結合抗体とＨＰＬＣ等を用いる方法。

先ず、例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、特定糖鎖結合蛋白質とを反応させ、糖鎖結合ＣＥＡ類を生成させる。次いで、この試料と、標識競合性ＣＥＡ類結合抗体とを反応させて下記の複合体を生成させる。

非結合ＣＥＡ類－標識競合性ＣＥＡ類結合抗体

次いで、この複合体と遊離の標識競合性ＣＥＡ類結合抗体とを、適当な充填剤を充填したＨＰＬＣや電気泳動法等を用いて分離し、該複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の非結合ＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）とＣＥＡ類濃度との関係を表す検量線等に当てはめること等により、試料中の非結合ＣＥＡ類量を求めることができる。

尚、当然のことながら、特定糖鎖構造を有するＣＥＡ類（糖鎖結合ＣＥＡ類）量は、総ＣＥＡ類量から特定糖鎖構造以外の糖鎖構造を有するＣＥＡ類（非結合ＣＥＡ類）量を差し引くことによっても求めることができるし、特定糖鎖構造以外の糖鎖構造を有するＣＥＡ類（非結合ＣＥＡ類）量は、総ＣＥＡ類量から特定糖鎖構造を有するＣＥＡ類（糖鎖結合ＣＥＡ類）量を差し引くことによっても求めることができる。

【００２６】

ＩＩ．測定対象を一度の測定操作で測定する方法。

ＩＩ－１．標識ＣＥＡ類結合抗体と特定糖鎖結合蛋白質を用いる方法

特開平7-191027号公報に開示された方法に準じて以下の如く行えばよい。

即ち、例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、標識ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質とを反応させて、以下の複合体を形成させる。

標識ＣＥＡ類結合抗体－ＣＥＡ類

標識ＣＥＡ類結合抗体－ＣＥＡ類－特定糖鎖結合蛋白質

次いで、これら複合体並びに遊離の標識ＣＥＡ類結合抗体を、適当な充填剤を充填したＨＰＬＣや電気泳動法等を用いて夫々分離し、夫々の複合体中の標識

物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の、特定糖鎖構造を有するＣＥＡ類又は／及びそれ以外の糖鎖構造を有するＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）と各種ＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の特定糖鎖構造を有するＣＥＡ類及びそれ以外の糖鎖構造を有するＣＥＡ類、並びにこれらＣＥＡ類の合計、即ち総ＣＥＡ類を一度の測定で求めることができる。

尚、複合体並びに遊離の標識ＣＥＡ類結合抗体を分離する方法としては、操作性や繰り返し使用できること等を考慮するとＨＰＬＣを用いる方法が好ましい。

また、使用するＣＥＡ類結合抗体は、非競合性のものが好ましい。

【００２７】

ＩＩ－２．非競合性ＣＥＡ類結合抗体、競合性ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質とを用いる方法

先ず、例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、適当な標識物質が結合した非競合性ＣＥＡ類結合抗体（以下、「標識非競合性ＣＥＡ類結合抗体」と略記する。）、競合性ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質とを反応させて、以下の複合体を形成させる。

標識非競合性ＣＥＡ類結合抗体－ＣＥＡ類－競合性ＣＥＡ類結合抗体

標識非競合性ＣＥＡ類結合抗体－ＣＥＡ類－特定糖鎖結合蛋白質

次いで、これら複合体並びに遊離の標識非競合性ＣＥＡ類結合抗体を、適当な充填剤を充填したＨＰＬＣや電気泳動法等を用いて分離し、夫々の複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の、特定糖鎖構造を有するＣＥＡ類又は／及びそれ以外の糖鎖構造を有するＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質量（測定値）と各種ＣＥＡ類濃度との関係を表す検量線等に当てはめる等することにより、試料中の特定糖鎖構造を有するＣＥＡ類及びそれ以外の糖鎖構造を有するＣＥＡ類、並びにこれらＣＥＡ類の合計、即ち総ＣＥＡ類を一度の測定で求めることができる。

尚、複合体並びに遊離の標識非競合性ＣＥＡ類結合抗体を分離する方法として

は、操作性や繰り返し使用できること等を考慮するとＨＰＬＣを用いる方法が好ましい。

【００２８】

ＩＩ－３．標識ＣＥＡ類結合抗体、競合性ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質を用いる方法

先ず、①例えば血漿、血清、髄液、各種生体組織の成分抽出液、糞便、尿等の生体由来試料と、標識ＣＥＡ類結合抗体を反応させた後、当該反応液にさらに特定糖鎖結合蛋白質及び競合性ＣＥＡ類結合抗体を反応させて、以下の複合体を形成させる。

標識ＣＥＡ類結合抗体－ＣＥＡ類－競合性ＣＥＡ類結合抗体

標識ＣＥＡ類結合抗体－ＣＥＡ類－特定糖鎖結合蛋白質

次いで、これら複合体並びに遊離の標識ＣＥＡ類結合抗体を、適当な充填剤を充填したＨＰＬＣや電気泳動法等を用いて夫々分離し、夫々の複合体中の標識物質量を適当な方法により測定し、得られた測定値を、例えば予め濃度既知の、特定糖鎖構造を有するＣＥＡ類又は／及びそれ以外の糖鎖構造を有するＣＥＡ類を含む標準液を用いて同様の方法により測定を行って得られた、標識物質の測定値と各種ＣＥＡ類濃度との関係を表す検量線等に当てはめることにより、試料中の特定糖鎖構造を有するＣＥＡ類及びそれ以外の糖鎖構造を有するＣＥＡ類、並びにこれらＣＥＡ類の合計、即ち総ＣＥＡ類を一度の測定で求めることができる。さらに標識ＣＥＡ類結合抗体の反応後に、標識ＣＥＡ類結合抗体とエピトープの違うＣＥＡ類結合抗体を反応させることにより、複合体と、測定に影響する血清成分との性質の違いを大きくすることができるので、血清成分の影響が少なくなり、測定精度が向上するため好ましい。

尚、複合体並びに遊離の標識ＣＥＡ類結合抗体を分離する方法としては、操作性や繰り返し使用できること等を考慮するとＨＰＬＣを用いる方法が好ましい。

【００２９】

ＣＥＡ類結合抗体を用いる自体公知のＣＥＡ類測定法は、例えばＣＥＡ類結合抗体として抗ＣＥＡ類抗体を用いて、いわゆる酵素免疫測定法（EIA）、放射免疫測定法（RIA）、ＥＬＩＳＡ、蛍光免疫測定法（FIA）、ＨＰＬＣを用いる測定

方法（特開平9-301995号公報等）等の免疫学的測定法に準じて行えばよい。また、その測定原理も、サンドイッチ法、競合法、二抗体法等のいずれにてもよい。

種々のＣＥＡ類結合抗体や特定糖鎖結合蛋白質を固定化するために用いられる不溶性担体としては、上記した如き免疫学的測定法の分野で通常用いられるものでよく、例えば金属、ガラス、セラミック、シリコンラバー、例えばポリスチレン，ポリ塩化ビニル，ポリプロピレン，アクリル，ポリメチルメタクリレート等の合成高分子等で調製された、ビーズ、チューブ、多数のチューブが一体成形された専用のトレイ、マイクロタイタープレート等が挙げられ、固定化方法としても、上記した如き免疫学的測定法の分野で通常用いられる、例えば物理的吸着法、化学的結合法等が挙げられる。

【００３０】

本発明に係るＣＥＡ類結合抗体や特定糖鎖結合蛋白質に結合させる標識物質としては、例えばＥＩＡに於いて用いられるアルカリホスファターゼ，β-ガラクトシダーゼ，ペルオキシダーゼ（ＰＯＤ），マイクロペルオキシダーゼ，グルコースオキシダーゼ，グルコース-6-リン酸脱水素酵素，アセチルコリンエステラーゼ，リンゴ酸脱水素酵素，ルシフェラーゼ等の酵素類、例えばＲＩＡで用いられる^{99m}Tc，^{131}Ｉ，^{125}Ｉ，^{14}Ｃ，^{3}Ｈ等の放射性同位元素、例えばＦＩＡで用いられるフルオレセイン，ダンシル，フルオレスカミン，クマリン，ユーロピウム，ナフチルアミン或はこれらの誘導体等の蛍光性物質、例えばルシフェリン，イソルミノール，ルミノール，ビス(2,4,6-トリフロロフェニル)オキザレート等の発光性物質、例えばフェノール，ナフトール，アントラセン或はこれらの誘導体等の紫外部に吸収を有する物質、例えば4-アミノ-2,2,6,6-テトラメチルピペリジン-1-オキシル，3-アミノ-2,2,5,5-テトラメチルピロリジン-1-オキシル，2,6-ジ-t- ブチル-α-(3,5-ジ-t-ブチル-4-オキソ-2,5-シクロヘキサジエン-1-イリデン)-p-トリルオキシル等のオキシル基を有する化合物に代表されるスピンラベル化剤 としての性質を有する物質等が挙げられる。

【００３１】

また、上記した如き標識物質を、ＣＥＡ類結合抗体や特定糖鎖結合蛋白質に結合させる（標識する）方法としては、自体公知のＥＩＡ、ＲＩＡ或はＦＩＡ等に

於いて一般に行われている自体公知の標識方法に準じて行えばよい。また、標識方法として、アビジン（又はストレプトアビジン）とビオチンの反応を利用した常法を利用しても良い。

【００３２】

本発明のＨＰＬＣを利用する測定法に於いて用いられるＨＰＬＣ用装置も、通常この分野で用いられるものであればよい。

本発明のＨＰＬＣを利用する測定法に於いては、複合体と遊離の標識ＣＥＡ類結合抗体（或いは標識特定糖鎖結合蛋白質）とをより明確に分離するために、例えば特開平７－１９１０２７号公報、特開平9-301995号公報等に開示された、これらの分離を向上させるための物質（以下、「分離向上物質」と略記する。）を結合させた、ＣＥＡ類結合抗体や特定糖鎖結合蛋白質等を用いてもよい。

【００３３】

このような目的に用いられる分離向上物質としては、例えばα－キモトリプシノーゲン，β－ガラクトシダーゼ，リゾチーム，チトクロームＣ，トリプシンインヒビター等の蛋白質、例えばフェニルアラニン，プロリン，アルギニン，リジン，アスパラギン酸，グルタミン酸等のアミノ酸を含むペプチド、例えば臭素，塩素，沃素等のハロゲン原子、例えばポリエチレングリコール等の合成高分子、例えばポリグルタミン酸，ポリアスパラギン酸，ポリリジン，ポリアルギニン，ポリフェニルアラニン，ポリチロシン等のポリアミノ酸、炭素数３～10のアルキル鎖、例えばパルミチン酸，オレイン酸，ステアリン酸等の脂肪酸、例えばN-(ε-マレイミドカプロイルオキシ)スクシンイミド [N-(ε-maleimidocaproyloxy) succinimide] (EMCS),N-スクシンイミヂル-6-マレイミドヘキサノエイト (N-Succinimidyl-6-maleimidohexanoate)，ビスマレイミドヘ キサン (Bismaleimidohexane) (BMH)，オクチルアミン等のＣＥＡ類結合抗体や特定糖鎖結合蛋白質に結合し得る反応基を有し且つ疎水性若しくはイオン性を有する化学物質、例えば特開平9-301995号公報に記載された4-(p-マレイミドフェニル) ブチリルAla-(Tyr(SO_3H))$_5$、4-(p-マレイミドフェニル) ブチリルAla-(Tyr(SO_3H))$_8$等の強酸残基含有ペプチド等が好ましく挙げられる。尚、分離向上物質は、測定対象であるＣＥＡ類、ＣＥＡ類結合抗体、特定糖鎖結合蛋白質の性質（例えばｐＨ安定性，疎水

度，水溶液への溶解度，等電点等）を考慮した上で適宜選択して用いればよい。

【００３４】

分離向上物質と、ＣＥＡ類結合抗体又は／及び特定糖鎖結合蛋白質との結合方法も、（１）自体公知のＥＩＡ、ＲＩＡ或いはＦＩＡ等において一般に行われている自体公知の標識物質と抗体との結合方法（例えば、医学実験口座、第８巻、山村雄一監修、第１版、中山書店、1971；図説　蛍光抗体、川生明著、第１版、(株)ソフトサイエンス社、1983；酵素免疫測定法、石川栄治、河合忠、宮井潔編、第２版、医学書院、1982、等）、（２）自体公知の物質の修飾および結合方法（例えば、蛋白質の化学修飾〈上〉〈下〉、瓜谷郁三、志村憲助、中村道徳、船津勝編集、第１版、(株)学会出版センター、1981；ポリエチレングリコール修飾蛋白質、稲田祐二他、生化学、第62巻、第11号、P1351-1362、（社）日本生化学会、1990；DNA PROBES, George H.K. and Mark M.M. STOCKTON PRESS,1989 、等）等に準じて行えばよい。

【００３５】

本発明の測定方法により得られた、総ＣＥＡ類、特定の糖鎖構造を有するＣＥＡ類、それ以外の糖鎖構造を有するＣＥＡ類等の値を適宜組み合わせることにより癌の判定を行うことが可能である。

また、種々の異なる特定糖鎖結合蛋白質を用いて測定を行い、その結果を解析することにより、癌の種類（癌の存在部位）を判別することも可能となる。

【００３６】

本発明のＣＥＡ類の検出用キットは、上記した本発明の測定法に用いられるものであって、ＣＥＡ類結合抗体と特定糖鎖結合蛋白質を含有してなるものであり、その構成要素の好ましい態様と具体例は上で述べたとおりである。

【００３７】

当該キットには、通常この分野で用いられる試薬類、例えば緩衝剤、反応促進剤、糖類、蛋白質、塩類、界面活性剤等の安定化剤、防腐剤等であって、共存する試薬等の安定性を阻害したり、ＣＥＡ類と、ＣＥＡ類結合抗体又は／及び特定糖鎖結合蛋白質との反応を阻害しないものが含まれていてもよい。また、その濃度も、通常この分野で通常用いられる濃度範囲から適宜選択すればよい。

【００３８】

また、マグネシウム等の金属イオンがレクチン活性や安定性に影響を与えることはよく知られており、これらを含んでいてもよい。

【００３９】

本発明の試薬に於いて用いることのできる緩衝剤としては、例えばトリス緩衝剤、リン酸緩衝剤、ベロナール緩衝剤、ホウ酸緩衝剤、グッド緩衝剤等通常免疫比濁法、免疫比ろう法、ＲＩＡ、ＥＩＡに用いられている緩衝剤は全て挙げられ、測定反応時のｐＨとしては抗原抗体反応やＣＥＡ類とレクチン等との反応を抑制しない範囲であれば特に限定されないが、通常６～１０である。

【００４０】

以下に実施例を挙げて本発明を更に詳細に説明するが、本発明はこれら実施例により何等制限されるものではない。

【００４１】

【実施例】

実施例１　ＨＰＬＣによるＣＥＡ類糖鎖分別測定法

（ＰＯＤ標識ＣＥＡ類結合抗体）

抗ＣＥＡ抗体（和光純薬工業(株)製）をＣＥＡ類結合抗体として用い、これを常法によりＦab'とし、このＦab'とＰＯＤとを常法により結合させてＰＯＤ標識ＣＥＡ類結合抗体とした。

（試料）

ＣＥＡ（COSMO-Bio社製）を50mMリン酸緩衝液(pH6.5、0.9%NaCl,１% BSA含有)に溶解して1000ng／mlとしたものを用いた。

（ＣＥＡ類の糖鎖分別測定用分析機器類及び試薬）

以下の機器及び試薬を用いた。

（ＨＰＬＣ条件）

分析機器：高速液体クロマトグラフィー（ＬＣ－９Ａ　(株)島津製作所製）

分析カラム：Diol-300(φ8.0mm×300mm、和光純薬工業(株))、

ゲルろ過分画分子量　22,000～660,000

分析用溶離液：50mM PBS pH7.5

分析用基質液：25mM アセトアミドフェノール（15mMクエン酸緩衝液、pH5.5）

流速：溶離液１ml／min、基質液0.1ml／min

検出：Ex 328nm、Em 432nm

（分析方法）

① ＣＥＡ類結合抗体の溶出位置の確認

精製水30μlにＰＯＤ標識ＣＥＡ類結合抗体溶液30ul（抗体濃度：１×10^{-8}M）を加え、30℃で30分間インキュベーションした後、30μlをゲルろ過カラムに注入し、分離しながら、溶出液にオンラインで分析用基質液を添加し、60℃、30秒反応により生成する蛍光量を検出した。

②総ＣＥＡ類の溶出位置の確認

試料30μlにＰＯＤ標識ＣＥＡ類結合抗体溶液30ul（抗体濃度：１×10^{-8}M）を加え、30℃で30分間反応させた後、反応液の30μlをゲルろ過カラムに注入し、分離しながら、溶出液にオンラインで分析用基質液を添加、60℃、30秒反応により生成する蛍光量を検出した。

③糖鎖変異ＣＥＡ類の溶出位置の確認

試料30μlにＰＯＤ標識ＣＥＡ類結合抗体溶液30ul（抗体濃度：１×10^{-8}M）を加え、30℃で30分間反応させた。次いで特定糖鎖結合抗体であるＬｅ^{b}に対する抗体（Signet社製）含有50mMリン酸緩衝液(pH6.5,0.9%NaCl,1% BSA含有、抗体濃度：１×10^{-7}M）30μlを反応液に加え、30℃で30分間反応させた。 反応後、反応液の30μlをゲルろ過カラムに注入し、分離しながら、溶出液にオンラインで分析用基質液を添加し、60℃、30秒反応により生成する蛍光量を検出した。

【００４２】

（結果）

得られた高速液体クロマトグラフィー分析パターンを図１に示す。

図１(a)の結果から、遊離のＰＯＤ標識ＣＥＡ類結合抗体は保持時間約11分の位置に溶出することが判る。これに対し、ＰＯＤ標識ＣＥＡ類結合抗体と試料とを反応させた場合、ＣＥＡ類－ＰＯＤ標識ＣＥＡ類結合抗体の免疫複合体のピーク１が、保持時間約8.5分の位置に出現し（図1(b)）、更に特定糖鎖結合抗体を添加すると、ピーク１以外に、ＰＯＤ標識ＣＥＡ類結合抗体-ＣＥＡ類-特定糖鎖結

合抗体の免疫複合体のピーク２が保持時間約7.5分の位置に出現することが判る(図1(c))。また、抗Ｌｅ^{b}抗体の代わりに抗Ｌｅ^{y}抗体を用いて同様の実験を行っても、ＰＯＤ標識ＣＥＡ類結合抗体－ＣＥＡ類－特定糖鎖結合抗体の免疫複合体（ピーク２）が出現することが確認できた。

【００４３】

以上のことより、ＣＥＡ類結合抗体及び特定糖鎖結合蛋白質を用いる本発明の方法に依れば、特定の変異した糖鎖構造を有するＣＥＡ類とそれ以外の糖鎖構造を有するＣＥＡ類を分別測定できることが判る。

【００４４】

実施例２　本発明方法による癌の判別

（ＰＯＤ標識ＣＥＡ類結合抗体）

実施例１と同様にして調製した。

（試料）

癌患者由来血清９例、健常人由来血清4例を試料として用いた。

（各種ＣＥＡ類の分別測定）

実施例１と同じ機器、試薬を用い、以下のようにして行った。尚、ＨＰＬＣ条件も、実施例１と同様にして行った。

（分析方法）

① 試料30μlにＰＯＤ標識ＣＥＡ類結合抗体溶液30ul（抗体濃度：１×10^{-8}M）を加え、30℃で30分間反応させた。次いで抗糖鎖抗体であるS-Le^{a},S-Le^{x},Le^{a},Le^{y}のいずれかに対する抗体（抗S-Le^{a}抗体及び抗S-Le^{x}抗体は和光純薬工業(株)製、抗Le^{a}抗体及び抗Le^{y}抗体はSignet社製）含有50mMリン酸緩衝液(pH6.5,0.9%NaCl,１% BSA含有、抗体濃度：１×10^{-7}M）30μlを反応液に加え、更に30℃で30分間反応させた。

② 反応後、反応液の30μlをゲルろ過カラムに注入し、分離しながら、溶出液にオンラインで分析用基質液を添加、60℃、30秒反応により生成する蛍光量を検出し、各ピーク面積を測定した。

（CEA濃度・計算方法）

上記方法に従って得られた結果を下記の式に代入して、試料中の、総ＣＥＡ類

量に対する特定の糖鎖構造を有するＣＥＡ類の量比（％）を算出した。尚、ピーク１はＣＥＡ類-ＰＯＤ標識ＣＥＡ類結合抗体の免疫複合体のピークであり、ピーク２はＰＯＤ標識ＣＥＡ類結合抗体-ＣＥＡ類-特定糖鎖結合抗体の免疫複合体のピークである。

試料中の総CEA類量に対する、特定の糖鎖構造を有するCEA類の量比（％）

＝[ピーク２の面積／（ピーク１の面積＋ピーク２の面積）]×１００

得られた結果を表１に示す。

【００４５】

表１

検　体	総CEA量に対する，特定の抗糖鎖抗体に反応した(糖鎖の変異した)CEA類量比(%)			
	抗 S-Le^a	抗 S-Le^x	抗 Le^a	抗 Le^x
	%	%	%	%
直腸癌	1.6	—	1.1	—
結腸癌	—	—	—	2.0
肺癌	2.3	3.1	2.2	—
	—	—	2.2	—
肝癌	5.9	—	—	—
中咽頭癌	17.7	—	—	—
乳癌	—	3.3	—	5.4
子宮頸癌	8.1	6.4	—	—
骨髄リンパ節転移	—	1.2	—	—
健常人	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	—	—	—

－：検出されず

【００４６】

表１から明らかな如く、健常人の血清中からは、変異した糖鎖構造を持つＣＥＡ類は検出されなかった。一方、癌患者の血清中からは各種変異した糖鎖構造を持つＣＥＡ類が検出され、癌の症例により糖鎖構造の変異が異なることが確認された。例えば、直腸癌ではS-Le^{a}とLe^{a}が観察され、結腸癌ではLe^{y}が、肺癌ではS-Le^a,S-Le^x,Le^{a}が、肝癌及び中咽頭癌ではS-Le^{a}が、乳癌ではS-Le^{x}とLe^{y}が、子

宮頸癌ではS-Le^a,S-Le^{x}が、骨髄リンパ節転移ではS-Le^{x}が、それぞれ観察され、癌の種類によって、糖鎖構造の変異の様子が異なることが判る。

以上のことから、糖鎖構造の変異したＣＥＡ類の分別測定は、癌の判定に用いることができ、また、癌の種類を鑑別するのにも非常に有用であることが示唆された。

【００４７】

【発明の効果】

本発明は、容易に且つ簡便に糖鎖構造の変異したＣＥＡ類を分別測定できる方法及びこの測定結果に基づき癌を鑑別し得る方法並びに試薬を提供するものであり、本発明の測定方法により得られた各種ＣＥＡ類の測定値を適宜組み合わせて用いることにより、癌の判別をすることができる。

【００４８】

【図面の簡単な説明】

【図１】

実施例１で得られた高速液体クロマトグラフィー分析パターンを示し、図１(a)はペルオキシダーゼ（ＰＯＤ）標識癌胎児性抗原（ＣＥＡ）類結合抗体の溶出パターン、図１(b)はＣＥＡ類とＰＯＤ標識ＣＥＡ類結合抗体（ピーク１）の免疫複合体の溶出パターン、図１(c)はＣＥＡ類とＰＯＤ標識ＣＥＡ類結合抗体の免疫複合体（ピーク１）及びＰＯＤ標識ＣＥＡ類結合抗体とＣＥＡ類と特定糖鎖結合抗体との免疫複合体（ピーク２）の溶出パターンを夫々示す。

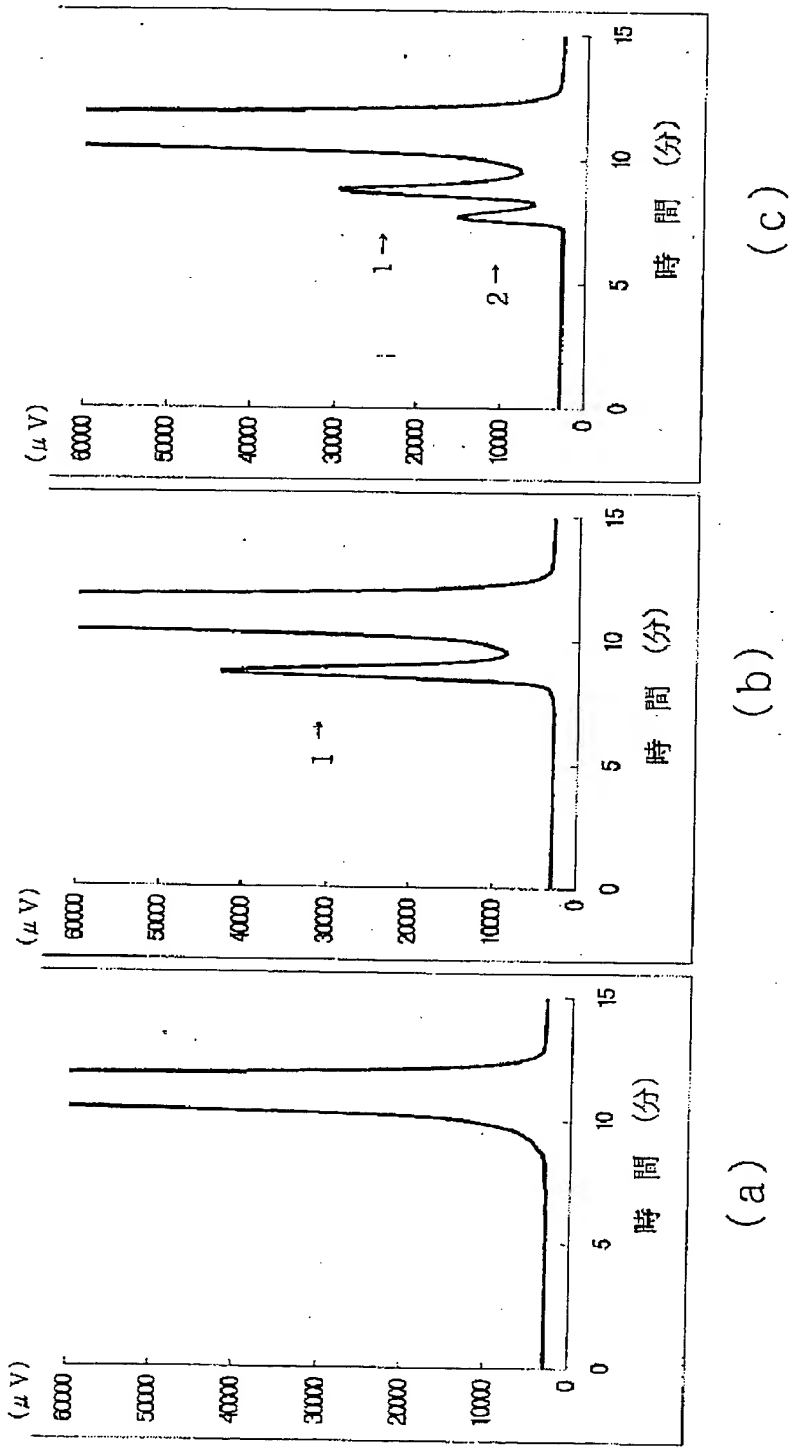
【符号の説明】

１：ピーク１

２：ピーク２

【書類名】　図面

【図１】



【書類名】　要約書

【要約】

【課題】　容易に且つ簡便に糖鎖構造の変異したＣＥＡ類を分別測定できる方法、この測定結果に基づき癌を鑑別し得る方法並びに試薬の提供。

【解決手段】　癌胎児性抗原（以下、ＣＥＡと略記する。）類の不変領域に特異的な抗体と、ＣＥＡ類の変異した糖鎖構造を認識する蛋白質とを用いることを特徴とする、糖鎖構造の変異したＣＥＡ類の検出方法、糖鎖構造の変異したＣＥＡ類の量に基づいて判定を行う、癌の判定方法、及びＣＥＡ類結合抗体と特定糖鎖結合蛋白質とを含有させて成る、糖鎖の変異したＣＥＡ類の検出用キット。

【選択図】　なし

出証特２０００－３０４８２０８

出　願　人　履　歴　情　報

識別番号　　〔０００２５２３００〕

１．変更年月日　　１９９０年　８月　７日
［変更理由］　新規登録
住　所　　大阪府大阪市中央区道修町３丁目１番２号
氏　名　　和光純薬工業株式会社

DECLARATION

I masahiko KOROKU, Wako Pure Chemical Industries, Ltd., Tokyo Office, 1-7, Nihonbashi honcho 2-vhome, Chuo-ku, Tokyo, Japan, do hereby solemnly declare:

1. That I am acquainted with the Japanese and English language; and
2. That the English text attached hereto is a true translation of the following document:

Japanese Patent Application No. 172485/1999

AND I MAKE SOLEMN DECLARATION conscientiously Believing the same to be true and correct.

This 25th day of July, 2000

Masahiko KOROKU

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: June 18, 1999

Application Number: No. 172485/1999

Application(s): Wako Pure Chemical Industries, Ltd.

June 23, 2000

Commissioner,
Patent Office Takahiko Kondo

Certificate No. 2000-3048208

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[FILING NUMBER BY APPLICANT]	H11P010
[FILING DATE]	June 18, 1999
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[DEPOSIT ACCOUNT NUMBER]	006035
[FEE (yen)]	21,000
[LIST OF ANNEXED DOCUMENT]	
[DOCUMENT]	Specification 1
[DOCUMENT]	Figure 1
[DOCUMENT]	Abstract 1
[REQUIREMENT OF PROOF]	Request

【Name of Document】 Specification

【Title of Invention】 A method for detection of carcinoembryonic antigens having a modified sugar chain structure

【Scope of Claim for a Patent】

【Claim 1】 A method for detection of carcinoembryonic antigens having a modified sugar chain structure which comprises using an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens.

【Claim 2】 The method according to Claim 1, wherein the detection is conducted by measuring an amount of a complex of carcinoembryonic antigens, an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens.

【Claim 3】 The method according to Claim 1, wherein the detection is conducted by

reacting a sample with an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens to give a complex of carcinoembryonic antigens, the specific antibody and the protein, and

measuring an amount of the complex.

【Claim 4】 A method according to any one of Claim 1 to 3, wherein the protein is an antibody or a lectin.

【Claim 5】 A method according to Claim 4, wherein the antibody is one recognizing a sugar chain containing a fucose residue and/or a sialic acid residue.

【Claim 6】 A method according to Claim 4, wherein the antibody is an anti-Lewis type sugar chain antibody or an anti-sialyl Lewis type sugar chain antibody.

【Claim 7】 A method according to Claim 6, wherein the anti-Lewis type sugar chain antibody is an anti-Le^a antibody, an anti-Le^b antibody, an anti-Le^x antibody or an anti-Le^y antibody.

【Claim 8】 A method according to Claim 6, wherein the anti-sialyl Lewis type sugar chain antibody is an anti-S-Le^a antibody or an anti-S-

Le^x antibody.

【Claim 9】 A method according to Claim 4, wherein the lectin is an L-fucose binding lectin, a D-galactose or an N-acetyl-D-galactosamine binding lectin, a D-mannose binding lectin, an N-acetylglucosamine binding lectin or a sialic acid binding lectin.

【Claim 10】 A method according to Claim 4, wherein the lectin is Concanavalin A, *Ricinus communis* agglutinin, *Lens culinaris* agglutinin or Phytohemagglutinin.

【Claim 11】 A method for detecting a cancer which comprises using an amount of a carcinoembryonic antigens having a modified sugar chain structure as an indicator for the detection.

【Claim 12】 The method according to Claim 11, wherein the detection is conducted by

measuring an amount of a complex of carcinoembryonic antigens, an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens, and

using the amount as an indicator for the detection.

【Claim 13】 The method according to Claim 11, wherein the detection is conducted by

measuring an amount of a complex of carcinoembryonic antigens, an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens, and an amount of a complex of carcinoembryonic antigens and the antibody, and

using the amounts as an indicator for the detection.

【Claim 14】 The method according to Claim 11, wherein the detection is conducted by

reacting a sample containing carcinoembryonic antigens with an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens to give a complex of carcinoembryonic antigens and the antibody and a complex of carcinoembryonic antigens, the antibody and the protein,

measuring an amount of these complexs, and

using the amounts as an indicator for the detection.

【Claim 15】 The method according to Claim 11, wherein the detection is conducted by

reacting a sample containing carcinoembryonic antigens with an antibody against a constant region of carcinoembryonic antigens and a protein capable of recognizing a modified sugar chain structure of carcinoembryonic antigens to give a complex of carcinoembryonic antigens and the antibody and a complex of carcinoembryonic antigens, the antibody and the protein,

measuring each independently an amount of these complexs,

calculating a ratio of the amount of the latter complex relative to a total amount of these complexs, and

using the ratio as an indicator for the detection.

【Claim 16】 A method according to any one of Claim 12 to 15, wherein the protein is an antibody or a lectin.

【Claim 17】 A method according to Claim 16, wherein the antibody is one recognizing a sugar chain containing fucose residue and/or a sialic acid residue.

【Claim 18】 A method according to Claim 16, wherein the antibody is an anti-Lewis type sugar chain antibody or an anti-sialyl Lewis type sugar chain antibody.

【Claim 19】 A method according to Claim 18, wherein the anti-Lewis type sugar chain antibody is an anti-Le^a antibody, an anti-Le^b antibody, an anti-Le^x antibody or an anti-Le^y antibody.

【Claim 20】 A method according to Claim 18, wherein the anti-sialyl Lewis type sugar chain antibody is an anti-S-Le^a antibody or an anti-S-Le^x antibody.

【Claim 21】 A method according to Claim 16, wherein the lectin is an L-fucose binding lectin, a D-galactose or an N-acetyl-D-galactosamine binding lectin, a D-mannose binding lectin, an N-acetylglucosamine binding lectin or a sialic acid binding lectin.

【Claim 22】 A method according to Claim 16, wherein the lectin is Concanavalin A, *Ricinus communis* agglutinin, *Lens culinaris* agglutinin or Phytohemagglutinin.

【Claim 23】 A kit for detection of carcinoembryonic antigens having a modified sugar chain structure which comprises an antibody against a constant region of carcinoembryonic antigens and a protein capable of

recognizing a modified sugar chain structure of carcinoembryonic antigens.

【Claim 24】 A kit according to Claim 23, wherein the protein is an antibody or a lectin.

【Claim 25】 A kit according to Claim 24, wherein the antibody is one recognizing a sugar chain containing a fucose residue and/or a sialic acid residue.

【Claim 26】 A kit according to Claim 24, wherein the antibody is an anti-Lewis type sugar chain antibody or an anti-sialyl Lewis type sugar chain antibody.

【Claim 27】 A kit according to Claim 26, wherein the anti-Lewis type sugar chain antibody is an anti-Le^a antibody, an anti-Le^b antibody, an anti-Le^x antibody or an anti-Le^y antibody.

【Claim 28】 A kit according to Claim 26, wherein the anti-sialyl Lewis type sugar chain antibody is an anti-S-Le^a antibody or an anti-S-Le^x antibody.

【Claim 29】 A kit according to Claim 24, wherein the lectin is an L-fucose binding lectin, a D-galactose or an N-acetyl-D-galactosamine binding lectin, a D-mannose binding lectin, an N-acetylglucosamine binding lectin or a sialic acid binding lectin.

【Claim 30】 A kit according to Claim 24, wherein the lectin is Concanavalin A, *Ricinus communis* agglutinin, *Lens culinaris* agglutinin or Phytohemagglutinin.

【Detailed Explanation of Invention】

【0001】

【Technical Field of Invention】

The present invention relates to a method for detection of carcinoembryonic antigens (hereinafter abbreviated as CEA), in particular, a method and kit for detection of CEAs having a modified sugar chain structure.

The present invention also relates to a method for detection of a cancer which comprises measuring an amount of CEAs having a modified sugar chain structure and detecting cancer on the basis of this amount.

【0002】

【Prior Art】

So-called carcinoembryonic antigen (hereinafter abbreviated as

CEA) is one of embryonic antigens originated from cancer cells, which is composed of a glycoprotein having a molecular weight of about 200,000 and a sugar content of about 50 %. CEA is produced from a surface of a normal mucosal cell of digestive organs and also from tumor tissues. It has been known that no CEA is found in blood of normal human beings but an amount of CEA is increased in blood and cells of human being suffering from organ cancers such as colorectal cancer, lung cancer, stomach cancer, breast cancer and liver cancer. Therefore, an amount of CEA has been useful as a marker for detecting various kinds of cancers, and measurement of an amount of CEA in blood has widely been used for screening of cancers, observation of progress of diseases after operation and prevention of recurrence of cancers. However, detection of cancers has been difficult to conduct, if the cancers are at rather early stage, only on the basis of the amount of CEA in blood.

Therefore, for the purpose of detection of cancer cells at rather early stage, an X-ray diagnosis, an endoscopy diagnosis etc. have been conducted. However, there have been such problems that detection of cancers at rather early stage is hardly possible by an X-ray diagnosis, and an endoscopy diagnosis is influenced by a level of a diagnosis technique and knowledge.

【0003】

On the other hand, CEAs from normal mucosal cells of digestive organs capable of producing CEAs, and those from tumor cells have been obtained separately in pure state, and analytical studies have been conducted on the sugar chain structures of those CEAs, whereby there has been found a difference in the sugar chain structures between CEAs from normal mucosal cells of digestive organs and those from cancer cells. And consequently use of those phenomena has been expected as useful for cancer diagnosis. (Katsuko Yamashita, J. Biol. Chem., 264 (30), 17873-17881(1989), Katsuko Yamashita, Glycobiology, 5 (1), 105-115 (1995)) Possible methods using this phenomena, however, would involve such problems that purification of CEAs and analysis of sugar chain structures thereof are necessarily required and therefore it takes a lot of time to conduct the necessary diagnosis.

【0004】

【Problems to be Solved by the Invention】

The present invention has been accomplished taking the above mentioned situation into consideration, and its object is to provide an easy and simple method for detection of CEAs in samples originated from a living body, to provide a method for detection of cancers on the basis of the CEAs amounts and also to provide a kit used in said methods.

【0005】

【Means for Solving Problems】

The present invention comprises the following features in order to attain the above-mentioned object.

(1) A method for detection of CEAs having a modified sugar chain structure which comprises using an antibody against a constant region of CEAs (hereinafter abbreviated as a CEAs binding antibody) and a protein capable of recognizing a modified sugar chain structure of CEAs (hereinafter abbreviated as a specific sugar chain binding protein).

(2) A method for detecting a cancer which comprises using an amount of a CEAs having a modified sugar chain structure as an indicator for the detection.

(3) A kit for detection of CEAs having a modified sugar chain structure which comprises a CEAs binding antibody and a specific sugar chain binding protein.

【0006】

Namely, the present inventors have earnestly investigated in order to solve the above mentioned theme to reach such a finding that the total amount of CEAs in a sample originated from a living body, an amount of CEAs having a specific modified sugar chain structure and/or an amount of CEAs having a sugar chain structure other than the specific one can be detected by using an antibody against a constant region of CEAs and a protein capable of recognizing a modified sugar chain structure of CEAs. And present inventors have further investigated to arrive at the finding that an amount of CEAs having the specific modified sugar chain structure or an amount of CEAs having a sugar chain structure other than the specific one, or a ratio of an amount of the CEAs having the specific modified sugar chain structure or a ratio of an amount of the CEAs having a sugar chain structure other than the specific one relative to the total amount of CEAs is useful, for example, for detection of colorectal cancer. The present invention has been accomplished on

the basis of these findings.

【0007】

The CEAs binding antibody of the present invention is not particularly limited and any antibody may be used so long as it is an antibody capable of specifically binding to a constant region of CEAs. The antibody may be either of the following polyclonal antibody or monoclonal antibody: e.g. a polyclonal antibody prepared by immunizing animals such as a horse, a cattle, a sheep, a rabbit, a goat, a rat, a mouse, etc. with CEAs, according to a conventional method, for example, the method described in Matsuhashi Tadashi et al. "Men-ekijikkengaku Nyumon" 2nd ed., GAKKAI-SHUPPAN CENTERLtd., 1981, etc.; and a monoclonal antibody produced by hybridomas obtained by fusing cells from a tumor line of a mouse together with spleen cells derived from a mouse immunized with CEAs, according to a conventional method, i.e., a cell fusion method established by G. Kohler and C. Milstein (Nature, 256, 495, 1975). These antibodies may be used singly or in proper combination of two or more thereof. In the present invention, "a constant region of CEAs" means a region common to all CEAs in body fluid (MASAHIDE KUROKI, HYBRIDOMA, 4 (11), 391-407 (1992)).

【0008】

The CEAs binding antibody includes an antibody having a property of binding to the constant region of CEAs but incapable of binding to CEAs having a modified sugar chain structure to which a protein capable of binding to a modified sugar chain structure (a specific sugar chain binding protein) is already bound (hereinafter abbreviated as a competitive CEAs binding antibody) and an antibody having a property of binding to all CEAs no matter whether a specific sugar chain binding protein is already bound or not (hereinafter abbreviated as a noncompetitive CEAs binding antibody).

【0009】

The specific sugar chain binding protein in the present invention includes, for example, an antibody and a lectin, which are capable of specifically binding to a specific sugar chain structure of CEAs. Examples of the specific sugar chain binding protein include those recognizing a sugar chain containing a fucose residue and/or a sialic acid residue, more specifically, an anti-Lewis type sugar chain antibody

capable of recognizing a Lewis type sugar chain structure such as an anti-Le^a antibody, an anti-Le^b antibody, an anti-Le^x antibody and an anti-Le^y antibody; an anti-sialyl Lewis type sugar chain antibody capable of recognizing a sialyl Lewis type sugar chain structure such as a S-Le^a and a S-Le^x; a L-fucose binding lectin such as *Lotus tetragonolobus* agglutinin; a D-galactose or N-acetyl-D-galactosamine binding lectin such as *Arachis hypogaea* agglutinin, soybean agglutinin, *Ricinus communis* agglutinin and Phytohemagglutinin; a D-mannose binding lectin such as Concanavalin A, *Lens culinaris* agglutinin and *Pisum sativum* agglutinin; a N-acetylglucosamine binding lectin such as Wheat germ agglutinin and *Datura stramonium* agglutinin; a sialic acid binding lectin such as *Limulus polyphemus* agglutinin. Among them, a D-galactose or N-acetyl-D-galactosamine binding lectin and a D-mannose binding lectin are preferable. These specific sugar chain binding proteins may be used singly or in proper combination of two or more thereof.

【0010】

In the above mentioned classification of lectins, "X binding lectin" means that a lectin which is once bound to a suitable sugar chain immobilized on an affinity column is easily eluted by "X". For example, "a D-galactose or N-acetyl-D-galactosamine binding lectin" means a lectin once bound to a suitable sugar chain immobilized on an affinity column is easily eluted by D-galactose or N-acetyl-D-galactosamine.

【0011】

The antibody capable of recognizing a modified sugar chain structure of CEAs may be either of a polyclonal antibody or a monoclonal antibody prepared in accordance with the above mentioned conventional methods.

【0012】

In the present invention, specific examples of the specific sugar chain are (1) those capable of binding to the above mentioned lectins, (2) those existing in the CEAs produced from a tumor cell such as colorectal cancer cells and the like. More specific examples are those described in Yamamoto, K., Eur. J. Biochem., 143 (1), 133-144, 1984, etc.

【0013】

A method for detection of CEAs having a modified sugar chain structure of the present invention is conduced by using of a CEAs binding antibody and a specific sugar chain binding protein in a proper combination, and measuring an amount of a complex of CEAs, the CEAs binding antibody and the specific sugar chain binding protein or confirming no such complex produced.

【0014】

A method for detecting a cancer of the present invention is conducted by detecting a cancer on the basis of an amount of CEAs having a modified sugar chain structure or an amount of other CEAs having a sugar chain structure other than the modified sugar chain structure. Specific examples of the methods are (1) a method comprising using an amount of a complex of CEAs, a CEAs binding antibody and a specific sugar chain binding protein measured by the above mentioned method as an indicator for the detection, (2) a method comprising using an amount of a complex (CEAs having a modified sugar chain structure) of CEAs, a CEAs binding antibody and a specific sugar chain binding protein and an amount of a complex (CEAs having a sugar chain structure other than the modified sugar chain structure) of CEAs and the CEAs binding antibody as an indicator for the detection, and (3) a method comprising measuring an amount of a complex (CEAs having a sugar chain structure other than the modified sugar chain structure) of CEAs and a CEAs binding antibody and an amount of a complex (CEAs having a modified sugar chain structure) of CEAs, the CEAs binding antibody and a specific sugar chain binding protein, calculating a ratio of the amount of the latter complex relative to the total amount of these complexes, in other words, calculating a ratio of CEAs forming the complexes together with a CEAs binding antibody and a specific sugar chain binding protein, and using the ratio as an indicator for the detection.

【0015】

Namely, in the present invention, specific examples of the concrete analyte(s) to be measured are total CEAs, CEAs having a modified sugar chain structure, CEAs having a sugar chain structure other than the modified sugar chain structure and the like. CEAs are decomposed into various fragments in a living body, and these fragments

are also included in the analyte to be measured of the present invention so long as it is one capable of binding to a CEAs binding antibody and/or a specific sugar chain binding protein. These analytes may be measured separately or simultaneously in one shot.

【0016】

In other words, a ratio of an amount of CEAs having a specific modified sugar chain structure or CEAs having a sugar chain structure other than the specific one relative to the total amount of CEAs, in a sample to be tested, is obtained by using a CEAs binding antibody and a specific sugar chain binding protein such as a lectin and an antibody mentioned above, and this ratio is compared with a ratio obtained by using a normal sample, whereby existence or non-existence of a cancer in the sample can be confirmed. The present invention has been completed on the basis of the above which has been found for the first time by the present inventors.

Specific examples of a method for measuring of CEAs are as follows.

【0017】

I. A method for each independently measuring analyte(s) to be measured

Analyte(s), namely, for example, total CEAs, CEAs having a specific modified sugar chain structure, CEAs having a sugar chain structure other than the specific one and the like can be measured respectively as follows.

【0018】

I-1. A measurement of the total CEAs

The measurement can be conducted according to a known method using a CEAs binding antibody.

【0019】

I-2. Measurement of CEAs having a specific modified sugar chain structure

I-2-1) A method using an insoluble carrier containing a CEAs binding antibody immobilized thereon

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a CEAs binding antibody immobilized on an

insoluble carrier to form the following immobilized complex.

[an insoluble carrier] — a CEAs binding antibody — CEAs

And then, after removing unnecessary coexisting substances by washing, the said immobilized complex is reacted with a specific sugar chain binding protein having a labeling substance bound thereto (hereinafter abbreviated as a labeled specific sugar chain binding protein) to form the following immobilized complex.

[an insoluble carrier] — a CEAs binding antibody — CEAs — a labeled specific sugar chain binding protein

After removing the free labeled specific sugar chain binding protein by washing the said immobilized complex, an amount of the labeling substance in the immobilized complex is measured by a suitable method, and an amount of CEAs having a specific modified sugar chain structure in the sample can be obtained by applying the obtained measurement value to a calibration curve showing the relationship between the labeling substance (measurement value) and the concentration of CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known concentrations of CEAs having the specific modified sugar chain structure.

【0020】

I-2-2) A method using an insoluble carrier containing a specific sugar chain binding protein immobilized thereon

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a specific sugar chain binding protein immobilized on an insoluble carrier to form the following immobilized complex.

[an insoluble carrier] — a specific sugar chain binding protein — CEAs

And then, after removing unnecessary coexisting substances by washing, the said immobilized complex is reacted with a CEAs binding antibody having a labeling substance bound thereto (hereinafter abbreviated as a labeled CEAs binding antibody) to form the following immobilized complex.

[an insoluble carrier] — a specific sugar chain binding protein — CEAs — a labeled CEAs binding antibody

After removing the free labeled CEAs binding antibody by washing the said immobilized complex , an amount of the labeling substance in the immobilized complex is measured by a suitable method, and an amount of CEAs having a specific modified sugar chain structure in the sample can be obtained by applying the obtained measurement value to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentration of CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known concentrations of CEAs having the specific modified sugar chain structure.

【0021】

I-2-3) A method using a labeled specific sugar chain binding protein and a high performance liquid chromatography (HPLC)

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a labeled specific sugar chain binding protein and a noncompetitive CEAs binding antibody to form the following immobilized complex.

a labeled specific sugar chain binding protein — CEAs — a noncompetitive CEAs binding antibody

And then, the said complex is separated from the free labeled specific sugar chain binding protein by using HPLC packed with a suitable carrier or an electrophoresis method, and an amount of the labeling substance in the complex is measured by a suitable method.

An amount of CEAs having a specific modified sugar chain structure in the sample can be obtained by applying the obtained measurement value to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentration of CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known concentrations of CEAs having the specific modified sugar chain structure.

【0022】

I-3. A measurement of CEAs having a sugar chain structure other than the specific one

I-3-1) A method using a free specific sugar chain binding protein

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a specific sugar chain binding protein to produce a complex of CEAs having a specific modified sugar chain structure and a specific sugar chain binding protein (hereinafter, if necessary, abbreviated as a binding protein bound CEAs).

And then, a sample containing only a CEAs having a sugar chain structure other than the specific one (hereinafter, if necessary, abbreviated as a nonbound CEAs) is obtained by separating the binding protein bound CEAs and the CEAs having no specific sugar chain binding protein bound thereto, in other words, a nonbound CEAs from the sample by using a known separating method such as a centrifuge method, a gel filtration method, a molecular fractionation membrane method and an electrophoresis method.

An amount of nonbound CEAs can be measured by measuring CEAs in the sample obtained above which contains only a nonbound CEAs by a known method using a CEAs binding antibody.

The sample containing only a nonbound CEAs may be prepared by treating the sample derived from a living body with an affinity chromatography using a carrier to which a specific sugar chain binding protein is bound.

【0023】

I-3-2) A method using a free specific sugar chain binding protein

First, a CEAs binding antibody immobilized on an insoluble carrier is reacted with a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine to form the following immobilized complex.

[an insoluble carrier] — CEAs binding antibody — CEAs

And then, after removing unnecessary coexisting substances by washing, the said immobilized complex is reacted with a specific sugar chain binding protein, and further reacted with a competitive CEAs binding antibody having a labeling substance bound thereto (hereinafter abbreviated as a labeled competitive CEAs binding antibody) to form the

following immobilized complexes.

[an insoluble carrier] — CEAs binding antibody — CEAs — a specific sugar chain binding protein

[an insoluble carrier] — CEAs binding antibody — CEAs — a labeled competitive CEAs binding antibody

After removing the free labeled competitive CEAs binding antibody by washing the said immobilized complexes, an amount of the labeling substance in the immobilized complexes is measured by a suitable method, and an amount of nonbound CEAs in the sample can be obtained by applying the obtained measurement value to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentration of CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known concentrations of CEAs having a sugar chain structure other than the specific one, i.e., nonbound CEAs.

【0024】

I-3-3) A method using an insoluble carrier having a competitive CEAs binding antibody immobilized thereon

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a specific sugar chain binding protein to produce a binding protein bound CEAs, and this sample is reacted with a competitive CEAs binding antibody immobilized on an insoluble carrier to form the following immobilized complex.

[an insoluble carrier] — a competitive CEAs binding antibody — a nonbound CEAs

And then, after removing unnecessary coexisting substances by washing, the said immobilized complex is reacted with a labeled CEAs binding antibody to form the following immobilized complex.

[an insoluble carrier] — a competitive CEAs binding antibody — a nonbound CEAs — a labeled CEAs binding antibody

After removing the free labeled CEAs binding antibody by washing the said immobilized complex, an amount of the labeling substance in the immobilized complex is measured by a suitable method, and an amount of nonbound CEAs in the sample can be obtained by applying the

obtained measurement value to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentration of CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known concentrations of nonbound CEAs.

【0025】

I-3-4) A method using a labeled competitive CEAs binding antibody and HPLC

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a specific sugar chain binding protein to produce a binding protein bound CEAs, and this sample is reacted with a labeled competitive CEAs binding antibody to form the following complex.

a nonbound CEAs — a labeled competitive CEAs binding antibody

And then, the said complex is separated from the free labeled competitive CEAs binding antibody by using HPLC packed with a suitable carrier or an electrophoresis method, and an amount of the labeling substance in the complex is measured by a suitable method.

An amount of nonbound CEAs in the sample can be obtained by applying the obtained measurement value to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentration of CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known concentration of nonbound CEAs.

In the above mentioned method, an amount of CEAs having a modified sugar chain structure (a binding protein bound CEAs) can also be obtained by subtracting the amount of CEAs having a sugar chain structure other than the specific one (a nonbound CEAs) from the amount of the total CEAs, and the amount of CEAs having a sugar chain structure other than the specific one (a nonbound CEAs) can be obtained by subtracting the amount of CEAs having a specific modified sugar chain structure (a binding protein bound CEAs) from the amount of the total CEAs.

【0026】

II. A method for measuring analyte(s) to be measured in one shot

II-1. A method using a labeled CEAs binding antibody and a

specific sugar chain binding protein

The measurement can be conducted as follows according to the method described in JP-A 7-191027 using a CEAs binding antibody.

Namely, first, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a labeled CEAs binding antibody and a specific sugar chain binding protein to produce the following complexes.

a labeled CEAs binding antibody — CEAs

a labeled CEAs binding antibody — CEAs — a specific sugar chain binding protein

And then, these complexes and a free labeled CEAs binding antibody are each independently separated from the sample by using HPLC packed with a suitable carrier or an electrophoresis method, and an amount of the labeling substance in the each complexes is measured by a suitable method.

Amounts of CEAs having a specific modified sugar chain structure and CEAs having a sugar chain structure other than the specific one, and the total amount of these CEAs, i.e., all CEAs can be obtained in one shot by applying the obtained measurement values to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentrations of various CEAs which has been previously obtained by carrying out the same measurement as described above expect for using standard solutions containing known CEAs having a specific modified sugar chain structure and/or CEAs having a sugar chain structure other than the specific one.

As the method for separating the complexes and free labeled CEAs binding antibody, a method using a HPLC is preferable, because it is easy to operate and possible to use repeatedly.

And as the CEAs binding antibody to be used, a noncompetitive one is preferable.

【0027】

II-2. A method using a noncompetitive CEAs binding antibody, a competitive CEAs binding antibody and a specific sugar chain binding protein

First, a sample derived from a living body such as plasma, serum,

cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a noncompetitive CEAs binding antibody having a suitable labeling substance bound thereto (hereinafter abbreviated as a labeled noncompetitive CEAs binding antibody), a competitive CEAs binding antibody and a specific sugar chain binding protein to form the following complexes.

a labeled noncompetitive CEAs binding antibody — CEAs — a competitive CEAs binding antibody

a labeled noncompetitive CEAs binding antibody — CEAs — a specific sugar chain binding protein

And then, these complexes and the free labeled noncompetitive CEAs binding antibody are each independently separated from the sample by using HPLC packed with a suitable carrier or an electrophoresis method, and an amount of the labeling substance in each of the complexes is measured by a suitable method.

CEAs having a specific modified sugar chain structure and CEAs having a sugar chain structure other than the specific one, and the total amount of these CEAs, i.e., all CEAs can be obtained in one shot by applying the obtained measurement values to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentrations of various CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known CEAs having a specific modified sugar chain structure and/or CEAs having a sugar chain structure other than the specific one.

As the method for separating the complexes and the free labeled noncompetitive CEAs binding antibody, a method using a HPLC is preferable, because it is easy to operate and possible to use repeatedly.

【0028】

II-3. A method using a labeled CEAs binding antibody, a competitive CEAs binding antibody and a specific sugar chain binding protein

First, a sample derived from a living body such as plasma, serum, cerebrospinal fluid, various extraction solutions from body tissues, feces, and urine is reacted with a labeled CEAs binding antibody, and after the reaction, the reaction solution is further reacted with a specific sugar

chain binding protein and a competitive CEAs binding antibody to form the following complexes.

a labeled CEAs binding antibody — CEAs — a competitive CEAs binding antibody

a labeled CEAs binding antibody — CEAs — a specific sugar chain binding protein

And then, these complexes and the free labeled CEAs binding antibody are each independently separated from the sample by using HPLC packed with a suitable carrier or an electrophoresis method, and an amount of the labeling substance in each of the complexes is measured by a suitable method.

CEAs having a specific modified sugar chain structure and CEAs having a sugar chain structure other than the specific one, and the total amount of these CEAs, i.e., all CEAs can be obtained in one shot by applying the obtained measurement values to a calibration curve showing the relationship between the labeling substance (measurement value) and a concentrations of various CEAs which has been previously obtained by carrying out the same measurement as described above except for using standard solutions containing known CEAs having a specific modified sugar chain structure and/or CEAs having a sugar chain structure other than the specific one.

Further, in the above mentioned method, it is preferable to react the reaction product of the labeled CEAs binding antibody, after the reaction, further with a CEAs binding antibody having an epitope different from that of a labeled CEAs binding antibody. According to the mentioned above, it is possible to make larger the difference between the property of the complex and that of the ingredients in the serum which influence upon the measurement, whereby the influence by the ingredients in the serum can be lowered, and thus more accurate measurement can be attained.

As the method for separating the complexes and the free labeled noncompetitive CEAs binding antibody, a method using a HPLC is preferable because it is easy to operate and possible to use repeatedly .

【0029】

The known method for measuring CEAs using a CEAs binding antibody can be conducted by using, for example, a CEAs binding

antibody as a anti-CEAs antibody, according to an immunological method such as so-called enzyme immunoassay (EIA), radio immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) and a method using a HPLC described in JP-A 9-301995 etc.

The measurement principle may be any of a Sandwich method, a competitive method, a double antibody method, etc.

As the insoluble carrier used to immobilize the various CEAs binding antibody or the specific sugar chain binding protein, there can be exemplified by the following ones which have usually been used in the field of the above mentioned immunological method: e.g. an insoluble carrier such as a bead, a tube, a tray formed with many tubes and a microtiterplate, which are prepared from a metal, a glass, a ceramic, a silicone rubber and synthetic polymers such as polystylene, polyvinyl chloride, polypropylene, acryl polymers and polymethylacrylate. As the method for immobilizing the various CEAs binding antibody or the specific sugar chain binding protein to the insoluble carrier, there can be exemplified by methods usually used in the field of the above mentioned immunological method such as a physical absorption method and a chemical absorption method.

【0030】

As the labeling substance to be bound to the CEAs binding antibody or the specific sugar chain binding protein in the present invention includes, for example, enzymes such as alkaline phosphatases, β-galactosidase, peroxidase (POD), micro-peroxidase, glucose oxidase, glucose-6-phosphate dehydrogenase, acetylcholine esterase, malate dehydrogenase and luciferase, which are used, for example, in enzyme immunoassay (EIA); radioisotopes such as ^{99m}Tc, ^{131}I, ^{125}I, ^{14}C, and ^{3}H, which have been used, for example, in radioimmunoassay (RIA); fluorescence substances such as fluorescein, dancyl, fluorescamine, cumarin, europium, naphthylamine and their derivatives, which have been used, for example, in fluoroimmunoassay (FIA); luminescent substances such as luciferin, isoluminol, luminol and bis(2,4,6-triflorophenyl) oxalate; substances having absorption in UV-ray region, such as phenol, naphthol, anthracene and their derivatives; and substances having spin-labeling properties, which are represented by compounds having an oxyl group, such as 4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl, 3-amino-2,2,5,5-

tetramethylpyrrolidin-1-oxyl and 2,6-di-t-butyl-α-(3,5-di-t-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyloxyl.

【0031】

As the method for binding the above mentioned labeling substances to the various CEAs binding antibody or the specific sugar chain binding protein, (i.e. a method for labeling the above mentioned labeling substances to the various CEAs binding antibody or the specific sugar chain binding protein), there can be exemplified by a known standard method usually used, for example, in conventional EIA, RIA and FIA.

As the labeling method, use may be made of a known method using a reaction of avidin (or streptoavidin) with biotin.

【0032】

As an apparatus to be used in the method of the present invention for using HPLC, there can be exemplified by one usually used in this field.

In the method of using HPLC of the present invention, in order to separate completely the complex from the free labeled CEAs binding antibody (or the free labeled specific sugar chain binding protein), the CEAs binding antibody or the specific sugar chain binding protein to which a substance to improve the separation effect (hereinafter abbreviated as a separation-improving substance) is bound can be used.

【0033】

The separation-improving substance to be used for such purpose includes, for example, proteins such as α-chymotrypsinogen, β-galactosidase, lysozyme, cytochrome C and trypsin inhibitors; peptides containing aminoacids such as phenylalanine, proline, arginine, lysin, aspartic acid and glutamic acid; halogen atoms such as bromine, chlorine and iodine; synthetic polymers such as polyethylene glycol; poly(amino acid)s such as poly(glutamic acid)s, poly(aspartic acid)s, polylysines, polyarginines, polyphenylalanines and polythyrosines; compounds containing alkyl chains having 3 to 10 carbon atoms such as fatty acids (e.g. palmitic acid, oleic acid, stearic acid, etc.); chemical substances which have a reactive group capable of binding to a CEAs binding antibody or a specific sugar chain binding protein and have hydrophobic or ionic properties such as N-(ε-maleimidocaproyloxy)succinimide (hereinafter abbreviated as EMCS), N-

succinimidyl-6-maleimidohexanoate, bismaleimidohexane (hereinafter abbreviated as BMH) and octylamine; peptides containing a strong acid residue described in JP-A 9-301995 such as 4-(p-maleimidophenyl)butyryl-alanine-(tyrosine(SO_3H))$_5$ and 4-(p-maleimidophenyl)butyryl-alanine-(tyrosine(SO_3H))$_8$. The separation-improving substance may be used by properly considering properties (e.g. pH stability, hydrophobicity, solubility in an aqueous solution, isoelectric point, etc.) of CEAs, the CEAs binding antibody and the specific sugar chain binding proteins as the analyte(s) to be measured.

【0034】

The method for binding the separation-improving substance to the CEAs binding antibody and/or the specific sugar chain binding protein can be conducted according to a known method such as (1) a known method for binding the labeling substance to the antibody usually used in a known EIA, RIA and FIA (e.g. Yuichi Yamamura "Igaku Jikken Koza Vol. 8" 1st ed.,NAKAYAMA-SHOTEN Ltd., 1971; Akira Kawano "Zusetsu Keikokotai" 1st ed., Soft Science, Inc., 1983; and Eiji Ishikawa, Tadashi Kawai and Kiyoshi Miyai "Koso Men-eki Sokuteiho" 2nd ed., IGAKU-SHOIN Ltd., 982), (2) a known method for modifying and binding a substance (e.g. Ikuzo Uritani, Kensuke Shimura, Michinori Nakamura and Masaru Funazu "Chemical Modification of Proteins Vols 1 and 2" 1st ed., GAKKAI-SHUPPAN CENTER Ltd., 1981; Yuji Inada et al. "Polyethylene Glycol-Modified Proteins" Seikagaku Vol. 62, No. 11, pp. 1351-1362, Japanese Biochemical Association, 1990; and Georg H. K. and Mark M. M. "DNA PROBES" STOCKTON PRESS, 1989).

【0035】

The detection of a cancer can be conducted by using, in proper combination, the total amount of CEAs, an amount of the CEAs having a specific modified sugar chain structure and an amount of the CEAs having a sugar chain structure other than the specific one, which are obtained by the present invention.

And further, a kind of a cancer (a region of existence of a cancer) can also be determined by conducting the measurement with the use of plural kinds of the specific sugar chain binding proteins and analyzing the results.

【0036】

The kit for detection of CEAs of the present invention is used for the above mentioned method of the present invention and comprises the CEAs binding antibody and the specific sugar chain binding protein. Preferable properties and specific examples of the main constituents of the kit are as described above.

【0037】

The kit may contain reagents usually used in the art, such as buffers, reaction accelerators, sugars, proteins, salts, stabilizers (e.g. surfactants) and antiseptics, so long as they do not decrease the stability of reagents and do not inhibit the reaction of CEAs, the CEAs binding antibody and/or the specific sugar chain binding protein. The concentrations of the reagents may be properly chosen from ranges usually employed in the art.

【0038】

The kit may contain metal ions such as magnesium ions which have been well known to affect the activity and stability of a lectin.

【0039】

As the buffers usable in the reagent or kit of the present invention, there can be exemplified by all buffers usually used in immunotubidimetry, immunonephelometry, RIA and EIA, such as Tris buffers, phosphate buffers, veronal buffers, borate buffers and Good's buffers. The pH upon the reaction for measurement is not particularly limited so long as it dose not inhibit the antigen-antibody reaction and the reaction of the CEAs with the lectin. The pH is generally in the range of 6 to 10.

【0040】

The present invention is explained below in further detail with reference to Examples, which are not by way of limitaion but by way of illustration.

【0041】

【Examples】

Example 1 Method for separately measuring the sugar chains of CEAs (POD labeled CEAs binding antibody)

Anti-CEA antibody (available from Wako Pure Chemical Industries, Ltd.) was used as a CEAs binding antibody. This antibody was made into Fab' by a conventional method, and this Fab' was

bound to POD by a conventional method, whereby a POD labeled CEAs binding antibody was obtained.

(Sample)

CEA (available from COSMO-Bio, Ltd.) was dissolved in 50 mM phosphate buffer (pH 6.5) containing 0.9 % NaCl and 1 % BSA to prepare a solution of a concentration of 1,000 ng/ml. The solution was used as a sample.

(Apparatus for analyzing and reagents for separately measuring the sugar chains of CEAs)

The following apparatus and reagents were used.

(HPLC conditions)

Apparatus for analyzing: HPLC (LC-9A, available from Shimazu Corporation.)

Column for analyzing: Diol-300, 8.0 mm in diameter × 300 mm (available from Wako Pure Chemical Industries, Ltd.), a molecular weight by gel filtration of 22,000 to 660,000

Eluent for analyzing: 50 mM PBS pH 7.5

Substrate solution for analyzing: 25 mM acetoamidophenol (15 mM citric acid buffer, pH 5.5)

Flow rate: eluent 1 ml/min., substrate solution 0.1 ml/min.

Detection: Ex 328 nm, Em 432 nm

(Analyzing procedure)

(1) Confirmation of an eluting position of the CEAs binding antibody

To 30 μl of pure water was added 30 μl of the POD labeled CEAs binding antibody solution (a concentration of antibody: 1×10^{-8} M), followed by incubating at 30 ℃ for 30 minutes. And then, 30 μl of thus obtained mixture was passed through a gel filtration column, and the substrate solution for the analysis was added to the effluent on-line to conduct a reaction at 60 ℃ for 30 seconds, and an amount of fluorescence produced by the reaction was detected.

(2) Confirmation of an eluting position of total CEAs

To 30 μl of the sample was added 30 μl of the POD labeled CEAs binding antibody solution (a concentration of antibody: 1×10^{-8} M), followed by allowing a reaction to take place at 30 ℃ for 30 minutes. And then, 30 μl of thus obtained reaction solution was passed through a

gel filtration column, and the substrate solution for the analysis was added to the effluent on-line to conduct a reaction at 60 ℃ for 30 seconds, and an amount of fluorescence produced by the reaction was detected.

(3) Confirmation of an eluting position of total CEAs having a specific modified sugar chain structure

To 30 μl of the sample was added 30 μl of the POD labeled CEAs binding antibody solution (a concentration of antibody: 1×10^{-8} M), followed by allowing a reaction to take place at 30 ℃ for 30 minutes. And then, 30 μl of 50 mM phosphate buffer solution (pH 6.5, a concentration of antibody: 1×10^{-7} M) containing an antibody against Le^b which was a specific sugar chain binding protein (available from Signet corporation), 0.9 % NaCl and 1 % BSA was added to the reaction solution, followed by allowing a reaction to take place at 30 ℃ for 30 minutes. After the reaction, 30 μl of thus obtained reaction solution was passed through a gel filtration column and, the substrate solution for the analysis was added to the effluent on-line to conduct a reaction at 60 ℃ for 30 seconds, and an amount of fluorescence produced by the reaction was detected.

【0042】

(Results)

Fig. 1 shows an analyzing pattern obtained by HPLC.

From the result of the Fig. 1 (a), it was found that the free POD labeled CEAs binding antibody was eluted at the position of retention time of about 11 minutes. On the other hand, when the POD labeled CEAs binding antibody was reacted with the sample, the peak 1 for a complex of the CEAs and the POD labeled CEAs binding antibody appeared at the position of retention time of about 8.5 minutes (Fig. 1 (b)), and when the specific sugar chain binding antibody was added to the above reaction product to cause a rection, the peak 2 for a complex of the POD labeled CEAs binding antibody, CEAs and the specific sugar chain binding antibody appeared at the position of retention time of about 7.5 minutes (Fig. 1 (c)) When the same experiment mentioned above was also conducted by using an anti-Le^y antibody in place of the anti-Le^b antibody, appearance of the peak 2 for a complex of the POD labeled CEAs binding antibody, CEAs and the specific sugar chain binding

antibody (peak 2) was confirmed.

【0043】

From the above, by the method of the present invention using the CEAs binding antibody and the specific sugar chain binding protein, it was found that the CEAs having a specific modified sugar chain structure and the CEAs having a sugar chain structure other than the specific one can be separately detected.

【0044】

Example 2 Detection of a cancer by the method of the present invention

(POD labeled CEAs binding antibody)

A POD labeled CEAs binding antibody was prepared by the same manner as Example 1.

(Samples)

As samples, there were used nine sera originated from cancer patients and four sera originated from normal human beings (normal sera).

(Method for separately measuring the various CEAs)

Using the same apparatus and reagents as Example 1, the following was conducted. The HPLC conditions were the same as in Example 1.

(Analyzing procedure)

(1) To 30 μl of the sample was added 30 μl of the POD labeled CEAs binding antibody solution (a concentration of antibody: 1×10^{-8} M), followed by allowing a raction to take place at 30 ℃ for 30 minutes. And then, 30 μl of 50 mM phosphate buffer solution (pH 6.5, a concentration of antibody: 1×10^{-7} M) containing an antibody against any one of S-Le^a, S-Le^x, Le^a and Le^y, which was a specific sugar chain protein (antibody) (an anti-S-Le^a antibody and an anti-S-Le^x antibody are available from Wako Pure Chemical Industries, Ltd., and an anti-Le^a antibody and an anti-Le^y antibody are available from Signet corporation), 0.9 % NaCl and 1 % BSA was added to the reaction solution, followed by allowing a reaction to take place at 30 ℃ for 30 minutes.

(2) After the reaction, 30 μl of thus obtained reaction solution was passed through a gel filtration column and, the substrate solution for the analysis was added to the effluent on-line to conduct a reaction at 60 ℃

for 30 seconds, and an amount of fluorescence produced by the reaction was detected.

(Concentration of CEAs and calculation method)

A ratio (%) of the amount of the CEAs having a specific modified sugar chain structure relative to the amount of total CEAs was calculated by applying the results obtained according to the above mentioned method to the following equation. In the equation, peak 1 was the peak for a complex of the CEAs and the POD labeled CEAs binding antibody, and peak 2 was the peak for a complex of the POD labeled CEAs binding antibody, CEAs and the specific sugar chain binding antibody.

A ratio (%) of the amount of the CEAs having a specific modified sugar chain structure relative to the amount of total CEAs = [a peak area for the peak 2 / (a peak area for the peak 1 + a peak area for the peak 2)] × 100

The results obtained were shown in Table 1.

【0045】

Table 1

Sample Origin	A ratio (%) of the amount of the CEAs reacted to a specific anti-sugar chain antibody (an amount of the CEAs having a specific modified sugar chain structure) relative to the amount of total CEAs			
	Anti-S-Le^a antibody	Anti-S-Le^x antibody	Anti-Le^a antibody	Anti-Le^y antibody
	%	%	%	%
Rectum cancer	1.6	–	1.1	–
Colon cancer	–	–	–	2.0
Lung cancers	2.3 –	3.1 –	2.2 2.2	– –
Liver cancer	5.9	–	–	–
Oropharyngeal Cancer	17.7	–	–	–
Breast cancer	–	3.3	–	5.4
Cervix uteri Cancer	8.1	6.4	–	–
Matastasis of bone marrow lymph node	–	1.2	–	–
Normal human Beings	– – – –	– – – –	– – – –	– – – –

–: not detected

【0046】

As is clear from Table 1, no CEAs having a specific modified sugar chain structure was detected from normal human beings. On the other hand, from sera originated from cancer patients, various CEAs having a modified sugar chain structure were detected, and it was confirmed that varieties of sugar chain structures differed among the kinds of cancers.

For example, in rectum cancer, S-Le^a and Le^a were detected, and in colon cancer, Le^y; in lung cancer, S-Le^a, S-Le^x and Le^a; in liver cancer and oropharyngeal cancer, S-Le^a; in breast cancer, S-Le^x and Le^y; in cervix uteri cancer, S-Le^a and S-Le^x; in matastasis of bone marrow lymph node, S-Le^x were detected respectively. Namely, it is found that the kinds of the modified sugar chain structures differ among the kinds of

cancers.

From the above, it is suggested that the method for separately measuring the various CEAs can be used for detecting cancers and is extremely useful for defining the kinds of cancers.

【0047】

【Effect of Invention】

The present invention provides an easy and simple method for separately measurement of CEAs having a modified sugar chain structure and a kit used in said method. By using, in proper combination, the measurement results on various CEAs obtained by the method of the present invention, existence or non-existence of cancers and the kinds of the cancers can be also detected.

【0048】

【Brief Explanation of Figures】

【Fig. 1】

Fig. 1 shows an analysis pattern of a sample which was obtained by a high performance liquid chromatography (HPLC) in Example 1. Fig. 1 (a) is an elution pattern of a POD labeled CEAs binding antibody, Fig. 1 (b) is an elution pattern of a complex of CEAs and a POD labeled CEAs binding antibody (peak 1), and Fig. 1 (c) is an elution pattern of a complex of CEAs and a POD labeled CEAs binding antibody (peak 1) and a complex of CEAs, a POD labeled CEAs binding antibody and a specific sugar chain binding protein (antibody) (peak 2).

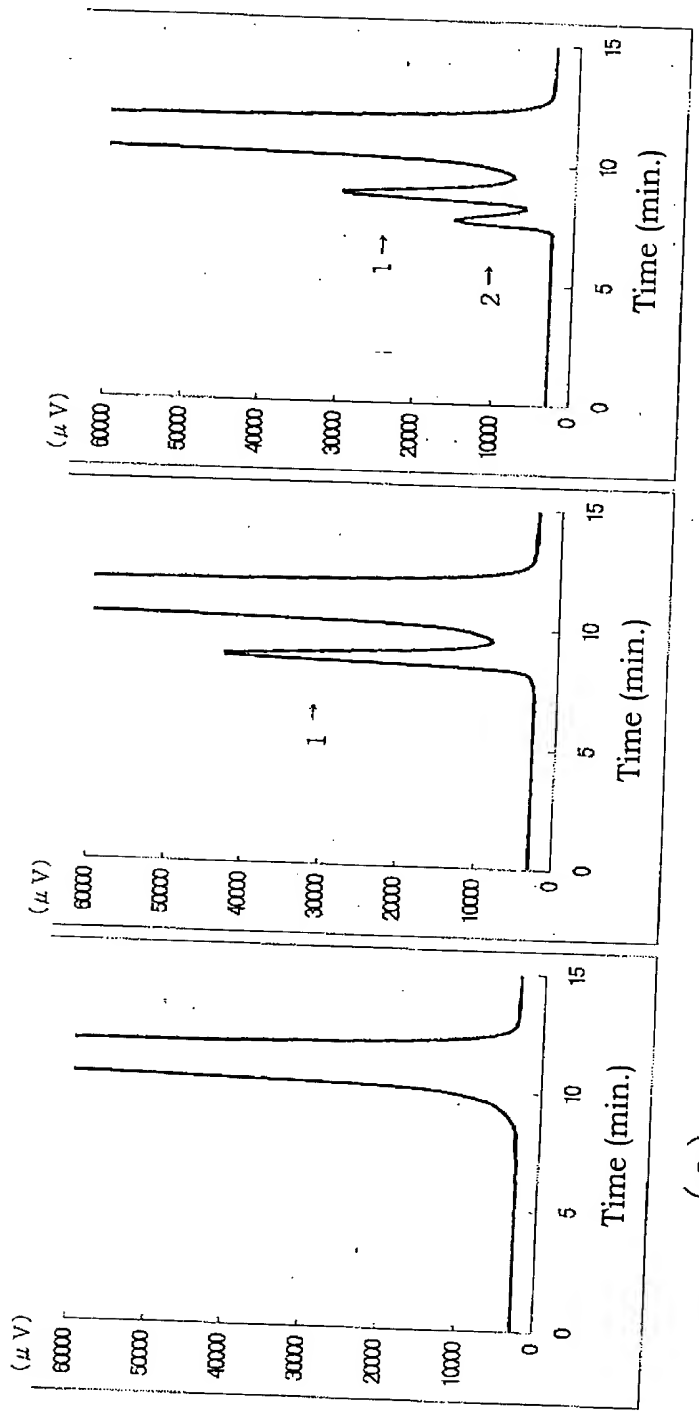
【Explanation of Symbols】

In Fig. 1, 1 shows peak 1.

In Fig. 1, 2 shows peak 2.

【Name of Document】 Figure

【Fig. 1】



【Name of Document】 Abstract

【Abstract】

【Problem】 To provide an easy and simple method for separately measurement of CEAs, to provide a method for detection of cancers on the basis of the CEAs amounts and also to provide a kit used in said methods.

【Solving means】 The present invention relates to a method for detection of carcinoembryonic antigens (hereinafter abbreviated as CEAs) having a modified sugar chain structure which comprises using an antibody against a constant region of CEAs and a protein capable of recognizing a modified sugar chain structure of CEAs, a method for detecting a cancer which comprises using an amount of CEAs having a modified sugar chain structure and a kit for detection of CEAs having a modified sugar chain structure which comprises an antibody against a constant region of CEAs and a protein capable of recognizing a modified sugar chain structure of CEAs.

【Selected Drawing】 None.

INFORMATION OF APPLICANT'S PERSONAL HISTORY

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